MMSL Military Medical Science Letters Since 1925

Mil. Med. Sci. Lett. (Voj. Zdrav. Listy) 2018, 87(supplementum 1)
ISSN 2571-113X (Online)

MEETING ABSTRACTS

BISTABLE DYNAMIC BEHAVIOR OF ENDOGENOUS BUTYRYLCHOLINESTERASE EXPRESSED IN Expi293 CELLS

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An endogenous tetrameric wild-type human BChE expressed in Expi293 cells hydrolyzes the neutral substrate N-methylindoxyl acetate (NMIA) with the same K_m as wild-type huBChE (0.14 mM) [1]. For this enzyme, the steady state is preceded by a pre-steady state phase of several minutes in 10 mM Bis-Tris, pH 7 at 25°C.
Thermal inactivation of this BChE is biphasic. Kinetic constants (k_1 and k_2) for thermal inactivation shows differences between this mutant and plasma derived wtBChE: the Expi293 is more stable at 55°C and less stable at 60°C than natural wtBChE [2]. At 55°C half-life times of the first and the second phases are 11 min and 43 min for plasma wtBChE; 14 min and 36 min for the Expi293 wtBChE, respectively. At 60°C, the corresponding values are 6 min and 14 min for natural wtBChE; 3 min and 11 min for Expi293 wtBChE. The endogenous enzyme is more stable in urea: urea-induced denaturation is 10 % slower than for the wtBChE and the urea concentration at the mid-point of denaturation is 4.1M for wtBChE and 4.6M for the endogenous enzyme.
A bistable dynamic behavior of the endogenous BChE is also observed from pre-steady state behavior for hydrolysis of 1 mM NMIA, showing either long *lags* or *bursts* under the same conditions while plasma BChE shows only *lags*. Molecular mechanic simulations have been undertaken to determine the molecular basis of bistability of this wild-typeBChE.

Acknowledgement

This work was supported by the Russian Science Foundation grant #17-14-01097 to PM.

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